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William J. Oostenink

Colgate University

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WILLIAM J. OOSTENINK[1]

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The development of the *Lychnis alba* ovary has been studied to provide original data regarding the rapid increase in ovary size which follows pollination. Preliminary study of the development of the ovary of an unpollinated flower has shown that this development follows a uniformly regular pattern quite unlike the rapid enlargement following pollination.

The development of the unpollinated flower's ovary was traced from the time it was 0.3mm in length to the time it had attained maturity at approximately 7.0mm in length. At this point natural abscission occurs if the flower is not pollinated.

A maximum ovary/fruit length of 15.2mm is attained within 48 hours following pollination. This represents an increase of 121.4% over the ovary length at the time of pollination. The presence of naturally occurring hormone(s) within the ovary whose presence (activation) is dependent primarily upon pollination and fertilization stimuli has been postulated as the reason for this size increase.

INDEX DESCRIPTORS: *Lychnis alba;* ovary development in Lychnis.

Dioecious plants have attracted the attention of botanists for many years. The segregation of sexes occuring in these plants is accompanied by an array of cytological, anatomical, morphological and physiological characteristics that continue to stimulate the interest of investigators. One such plant, *Lychnis alba* Mill. (*Melandrium album* Garcke), a member of the Caryophyllaceae, has been the subject of numerous studies. Genetic mechanisms have been of particular interest resulting in a voluminous literature dealing with sex-chromosomes, sex-determination, polyploidy and variational phenomena among others.

Often called the white cockle or campion, *Lychnis alba* is a long day plant found scattered along road sides, field borders and other waste places throughout wide areas of the northern hemisphere. The plant has been naturalized from Eurasia (Fernald, 1950).

An examination of the mature female flower of *Lychnis* prior to pollination reveals an ovary possessing a curiously swollen apex (Figure 1). If pollination occurs the ovary enlarges rapidly to more than twice the size it had attained at the time of pollination. Intrigued by the shape of this ovary and its rapid alteration following pollination/fertilization I undertook this study to describe the growth and development of the typical 5-styled *Lychnis alba* ovary with specific reference to the ovary wall and pericarp.

Although Brock (1931) and Ulvin (1930) have discussed the formation of the free central placenta in *Lychnis* no histological studies on the developing ovary wall or pericarp have previously been undertaken.

MATERIALS AND METHODS

All plants used in this study were greenhouse grown from seed collected near West Liberty, Iowa. Daylight was supplemented by artificial illumination, as necessary, to produce day lengths of 14 hours. Several hundred ovaries in various stages of development were collected from these plants. These were separated into 9 groups based on ovary length as determined by an eyepiece micrometer in a binocular dissecting microscope. The smallest ovary collected, representing the earliest stage of development studied, measured 0.3mm in length. The largest ovary, taken from a mature flower, was 7.0mm in length.

In a second collection approximately 150 flowers were hand pollinated and a number of these were collected at consecutive 12 hour intervals thereafter. Ovary/fruit which had been allowed to develop 12, 24, 36 and 48 hours following pollination was thus obtained. These spanned a size range of 7.5-15.2mm in length.

By selecting additional material, as required, all stages of ovary and fruit wall development were prepared for study. All tissue was killed and fixed in formalin-acetic acid-alcohol or Navashin's solution and was sectioned in paraffin. Sections cut 6μm thick were stained with safranin and fast green or hemalum. Lignin determinations were made using the phloroglucinol-HCl method.

Dean (1959) reported wide variation in the number of styles and other gynoecial components of *L. alba*. Most (66.9%) ovaries bear 5 styles and only those were used in this study.

For descriptive purposes all ovaries were divided transversely into 3 regions of equal size (Figure 2). Four lines superimposed on this figure indicate how 7 points of reference may be thus obtained. These are the lines themselves, points A,B,C,D, and the ovary regions A-B, B-C and C-D.

OBSERVATIONS AND DISCUSSION

The terms exocarp, mesocarp, and endocarp are conventionally used to describe the wall layers (pericarp) of a developing fruit. In this study the terms outer (epidermal layer), middle, and inner ovary wall will be used to designate the corresponding layers in the ovary of an unpollinated flower.

The smallest macroscopically recognizable *Lychnis* flower bud contains a developing ovary approximately 0.3mm in length (Figures 3,8). At this time the developing ovary walls (carpels) have not yet fused at their apex and little cellular differentiation is evident. The outer and inner ovary walls are each composed of a single layer of isodiametric cells. Cells in both layers are identical in size. Cells of the layers comprising the middle ovary wall are essentially the same size as those comprising the other wall layers but are much more irregular in shape. This region of the ovary wall also often exhibits an intricate system of intercellular air spaces somewhat resembling those of leaf mesophyll. Although these air spaces may persist throughout development, most mature ovaries studied did not possess them.

The ovary (carpel) walls fuse at their apex when the developing structure is approximately 0.6mm in length (Figures 4,9). The development of styles begins at this time and the initiation of ovules begins shortly thereafter (Figures 5,10 and 6,11). At this stage of development the outer and inner ovary wall cells remain

1. Department of Biology
Colgate University
Hamilton, New York 13346

OVARY DEVELOPMENT IN LYCHNIS

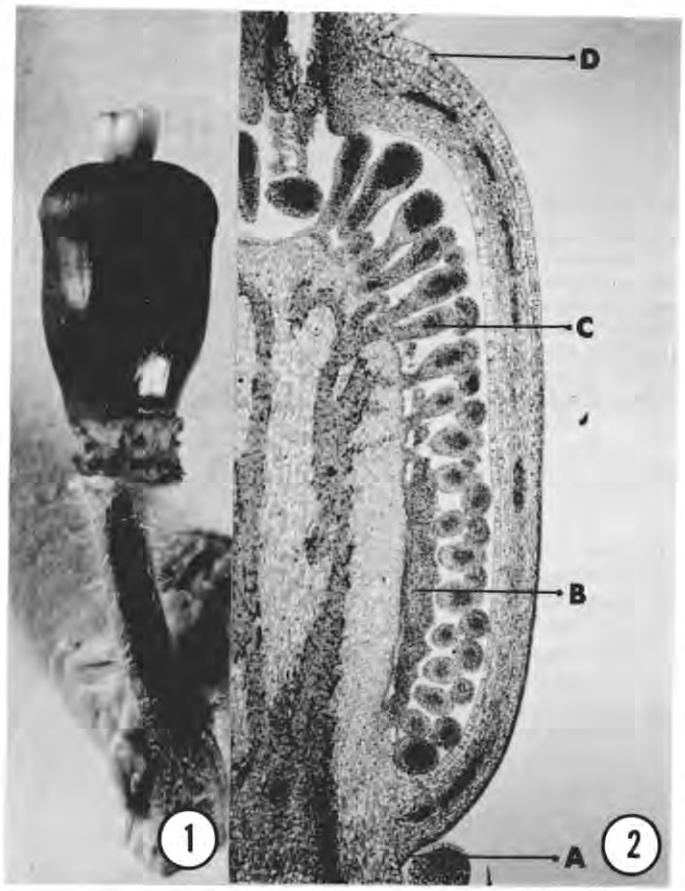


Figure 1
The mature unpollinated ovary of *Lychnis alba*.

Figure 2
A longitudinal section of a *Lychnis alba* ovary. Lines and letters divide ovaries into equal thirds.

isodiametric-cuboidal. Both of these wall layers also remain approximately the same size but are now somewhat smaller than those of the middle ovary wall due to a more rapid rate of enlargement of cells in that layer. The epidermal cells are filled with densely staining cytoplasm which contains a number of small scattered vacuoles. Nuclei in these epidermal cells are larger than those in cells of the other ovary wall layers.

A cuticle first becomes evident when the developing ovary is 0.7-0.8mm in length. It occurs on the exposed tangential surfaces of both inner and outer ovary wall cells. At ovary maturity the cuticle is approximately 1μm in thickness. As ovary maturity approaches cutin is replaced by lignin in the outer ovary wall cells near the ovary's apex.

Mitotic division figures occur frequently in all cell layers of the young ovary wall. During ovary development from 0.3mm to approximately 3.5mm in length, the incidence of cell division was nearly 10 times greater than during the next 3.0mm of growth. As the ovary nears maturity, cell division virtually ceases. Throughout ovary development the planes of cell division in the middle ovary wall are randomly oriented whereas those in the outer and inner ovary walls are all oriented anticlinally. Only as the ovary nears maturity do periclinal divisions occur in the cells of the outer ovary wall, and these only in cells near the point of style emergence.

Until an ovary has reached 1.5mm in length, the proportional size relationship existing between the three wall layers since the beginning of development remains nearly constant (Figures 3-12). During the next 1.5mm of growth (i.e. from 1.5-3.0mm in length), however, this relationship changes. Cells comprising the inner ovary wall retain their characteristic shape but they increase in size rapidly and uniformly until they are nearly three times the size of a middle ovary wall cell. The cells of the outer ovary wall also increase in size, sometimes even more dramatically than those of the inner wall, but they do so in a non-uniform manner resulting in the distinctive appearance illustrated in figure 2. At the base of the ovary (point A), cells of the outer ovary wall enlarge primarily anticlinally while those near point B enlarge very little and remain nearly isodiametric. From this point upward anticlinal elongation of outer ovary wall cells also occurs. This cellular enlargement appears to be tightly controlled. Moving from point B through areas B-C and C-D one observes that each succeeding cell in the outer ovary wall layer elongates fractions of a μm more than the preceding cell upon which it is storied. The progression is highly ordered and the resulting shape of the outer ovary wall as seen in longitudinal section is quite striking (Figures 14-28). Table 1 records average anticlinal lengths occurring in the outer ovary wall at points A-D at different stages in the development of the ovary.

TABLE 1. *Average Anticlinal Length of Outer Ovary Wall Cells in μm*

Ovary Length	Cell Length at Point Indicated			
	A	B	C	D
0.35	6.1	6.1	6.1	no fusion
0.66mm	9.6	9.6	9.6	9.6
1.40mm	11.6	11.6	11.6	11.6
3.19mm	20.8	18.9	45.0	83.2
4.43mm	32.0	24.2	57.8	96.0
5.16mm	44.8	30.1	64.1	96.0
6.78mm	52.1	35.9	79.2	99.9

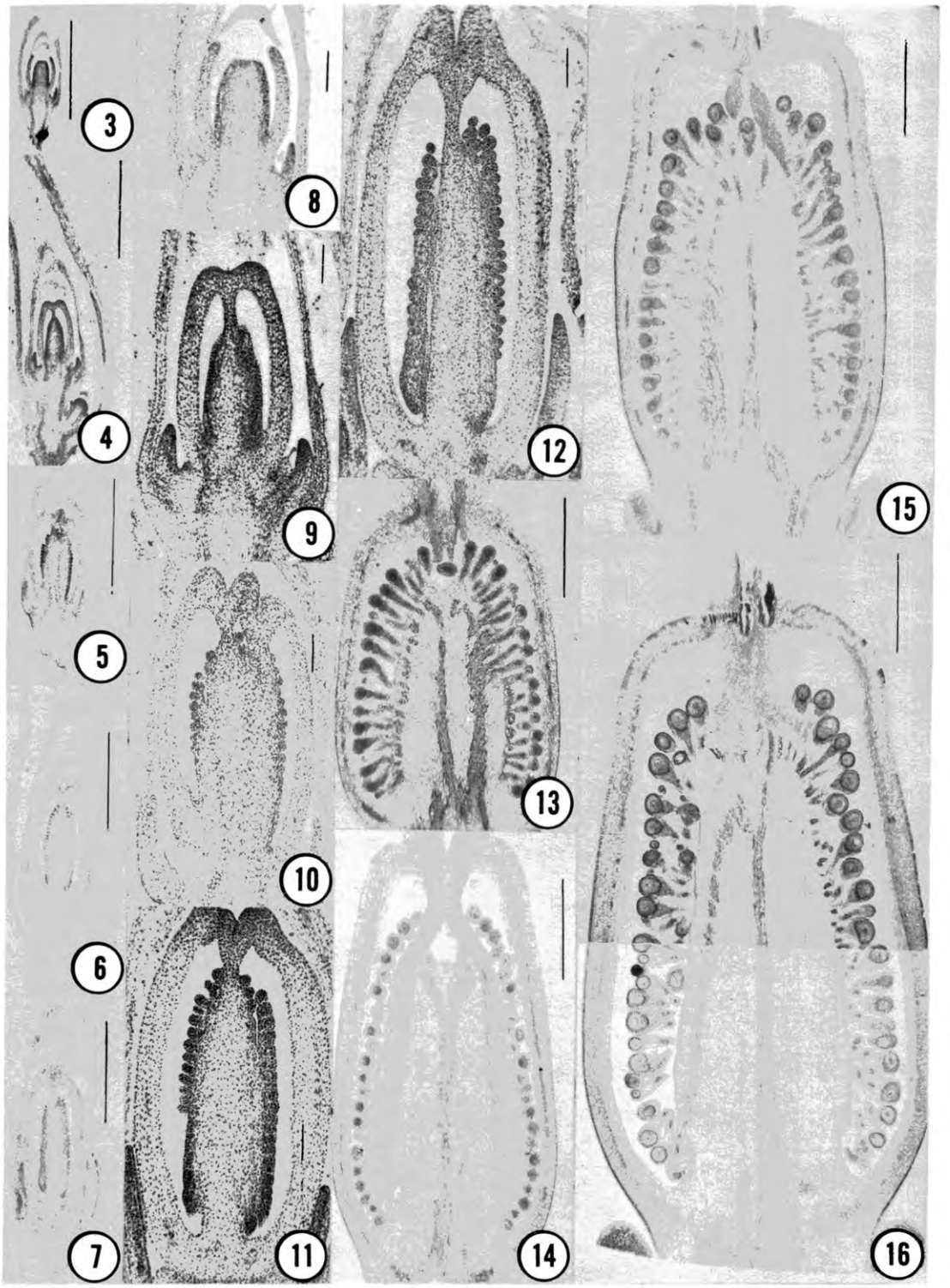
A final spatial relationship between the 3 wall layers is achieved by the time the developing ovary has reached 3.1mm in length. From this time until the mature size of the ovary has been reached, changes occurring within its wall mostly involve increases in cell size and two unusual modifications of outer ovary wall cells.

Beginning near point C, and extending to the level where styles emerge, the cells of the outer ovary wall become highly vacuolate and peculiarly lignified. Lignin is deposited on and/or in the outer tangential walls of these cells and "fingers" of lignin extend from this surface along the radial and transverse walls (Figures 20, 23, 26). Lignification becomes progressively heavier the nearer the cell is located to the ovary apex. Even the most heavily lignified cells, however, remain living. Chloroplasts occur in most cells of the outer ovary wall except those which contain lignin.

With the exception of that portion near point D, the outer ovary wall remains one cell layer in thickness. Periclinal divisions may occur near the ovary apex resulting in 2-3 layers of living cells all of whose walls contain some lignin.

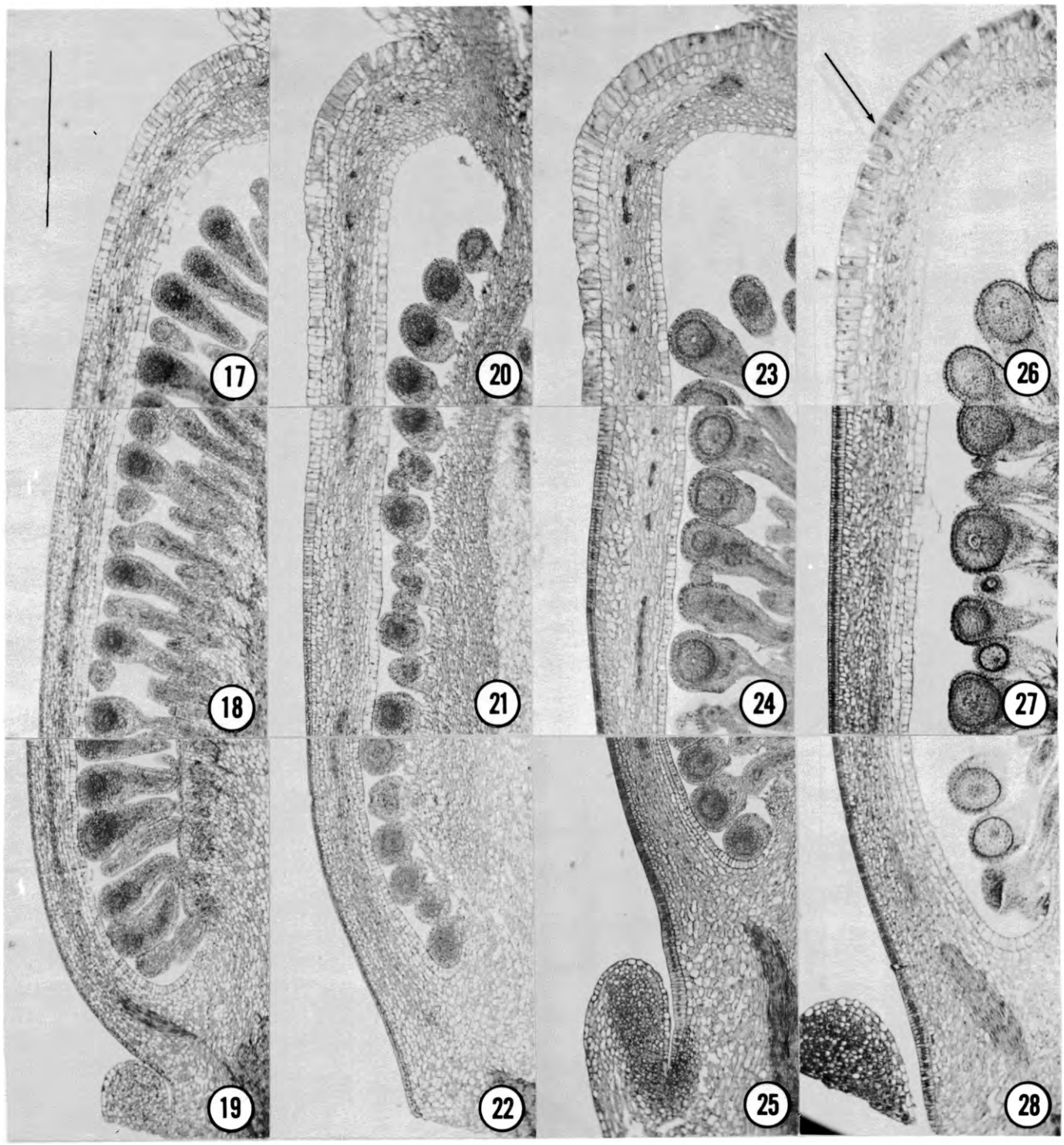
The mature capsule dehisces along 5 primary sutures which are identifiable even early in ovary development. Dehiscence mechanisms frequently involve the differing degree of rigidity existing between lignified and non-lignified tissues to produce areas of stress within a fruit which lead to its eventual opening. It is likely that the lignified regions of the *Lychnis* fruit exist for a similar purpose.

At maturity the much enlarged, lignified epidermal cells occurring in area C-D give the ovary a swollen appearance in that region. A lighter color is also imparted to this portion of the ovary due to the



Figures 3-16

Photomicrographs showing longitudinal sections of the ovary of unpollinated *Lychnis alba* flowers at various stages of development. Figures 8-12 are higher magnification duplications of figures 3-7. (3) at 0.3mm in length, (4) at 0.65mm in length, (5) at 0.8mm in length, (6) at 1.0mm in length, (7) at 1.4mm in length, (13) at 3.1mm in length, (14) at 4.4mm in length, (15) at 5.2mm in length, (16) at 6.8mm in length. Vertical scale markings on figs. 3-7 and 13-16 are 1.0mm in length, those on figs. 8-12 are 0.1mm in length.



Figures 17-28

Photomicrographs of longitudinal sections of the ovary of unpollinated *Lychnis alba* flowers showing ovary wall structure in areas indicated. (17-19) ovary length 3.1mm, (20-22) ovary length 4.4mm, (23-25) ovary length 5.2mm, (26-28) ovary length 6.8mm. Figures 17, 20, 23, 26 are of ovary areas C-D, figs. 18, 21, 24, 27 are of areas B-C and figs. 19, 22, 25, 28 are of areas A-B. The arrow on figure 26 indicates cell layer exhibiting peculiar "finger-like" lignification. Vertical scale markings are 0.5mm in length.

absence of chloroplasts in the lignified cells.

From the earliest stage of development studied (0.3mm ovary length), approximately 3 weeks is required for the ovary to reach its mature average length of nearly 7.0mm. When the ovary has matured, the flower opens at night and closes the following morning. The next night it will open once again and after closing the following morning, if it has not been pollinated, will begin to abscise (Devine, 1946). Prior to the completion of abscission the styles wither.

If pollination occurs, the ovary attains an average length of 7.5mm in 12 hours, an increase of 0.5mm over the average length of the mature unpollinated ovary. During this period the ovary retains the same basic shape it had at the time of pollination (Figure 29), although a few changes do occur in some of its cells.

In the developing exocarp, vacuolation becomes prominent (Figures 33-36), a good deal of starch is present, and numerous small granules of an undetermined nature are dispersed throughout the cytoplasm. Mesocarp cells remain relatively unchanged within areas A-B and B-C. In area C-D, however, the lignification noted earlier in the epidermal layer(s) of the unpollinated ovary now extends into the underlying (mesocarp) tissue. Before lignification begins, the cells in which that process will occur enlarge somewhat irregularly. The inflated appearance of the ovary's apex may consequently be accentuated temporarily. Endocarp cells are all heavily cutinized on the cell surface adjacent to the ovary's seed cavity. A few small vacuoles may be found in some of these cells but they do not appear to be common. As development proceeds, nuclei become progressively smaller in relation to cell size.

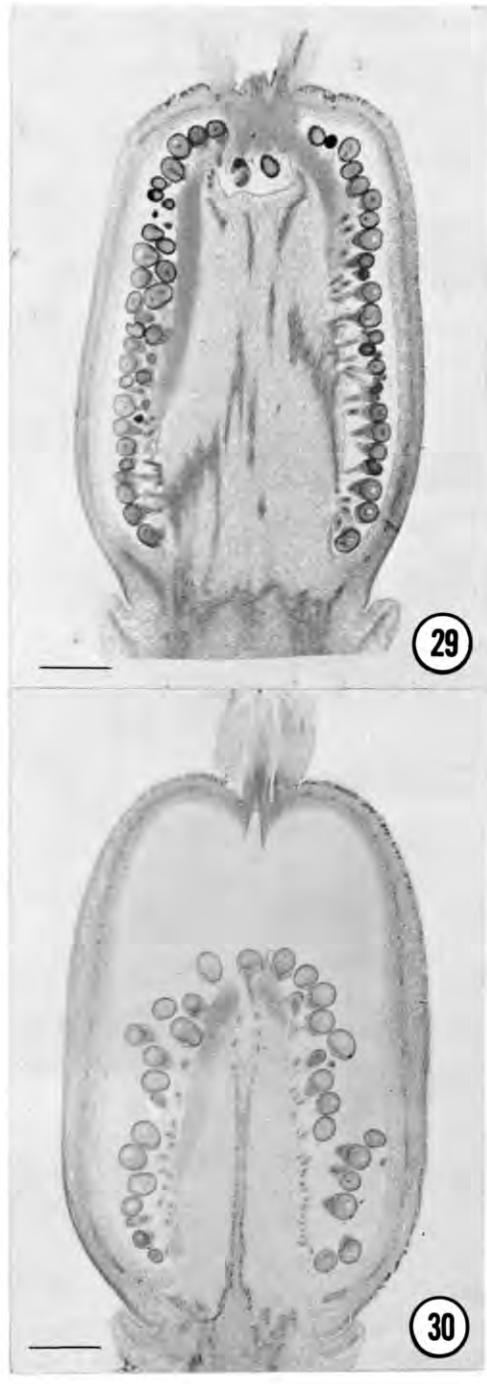
The preceding are the only changes that occur in cell contents during fruit development, which is, therefore, almost exclusively a matter of cell enlargement. Thirty-six hours after pollination, cell enlargement is conspicuous throughout the pericarp although not equally in all areas (Figures 41-44). Expansion of mesocarp cells within area C-D is by now considerably less than in other areas, quite probably due to their lignified walls. The entire pericarp in this region thus becomes markedly thinner than other fruit wall regions as development proceeds (Figures 30-32). Concomitantly the fruit rapidly loses the characteristic apical bulge it possessed at the beginning of development and acquires a broadly tear-drop shape.

A meticulous examination of nearly 6000 ovary/fruit sections at various stages of development following pollination failed to reveal a single division figure. The absence of such evidence of cell division throughout fruit development, a finding in keeping with Sinnott's (1939) work in the Cucurbitaceae among others, is one of the most striking characteristics of development. Whatever ultimate size the fruit attains is, therefore, attributable only to an increase in size of pre-existing cells and not to the appearance of new cells.

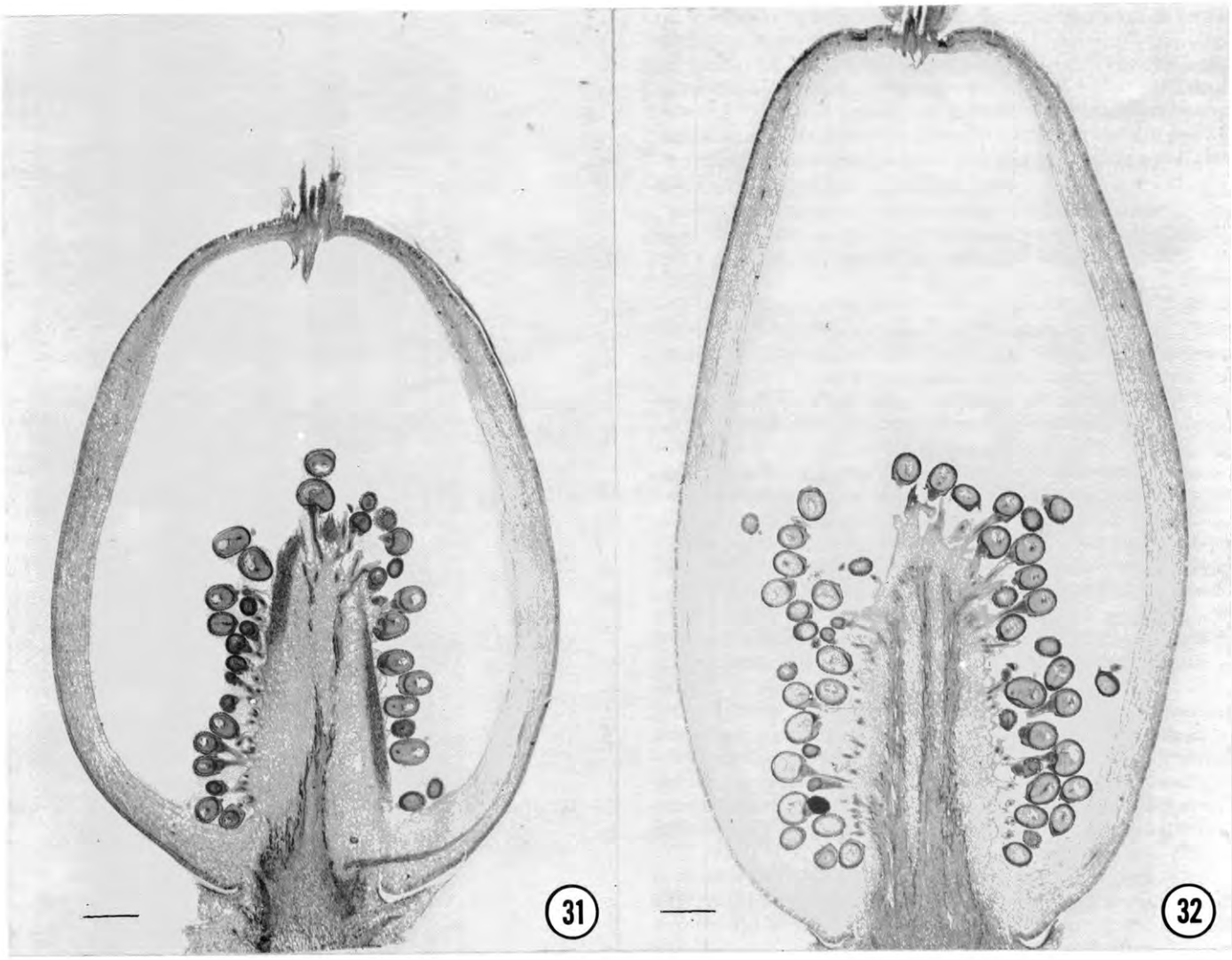
Fruit development, exclusive of the maturation of seeds, is completed 48 hours following pollination. Increases occurring in the length of the *Lychnis* fruit during that time are as follows (Table 2).

TABLE 2. *Increases in Ovary/Fruit Length at Intervals Following Pollination.*

Stage of Development	Length in Millimeters	Average % of Increase Over Length at Pollination
At Pollination	7.0	0.
12 hours after	7.5	7.1
24 hours after	8.0	14.2
36 hours after	11.5	64.2
48 hours after	15.2	121.4



Figures 29-30
Longitudinal sections of the ovary of pollinated *Lychnis alba* flowers. (29) 12 hours after pollination, (30) 24 hours after pollination. Horizontal scale markings are 1.0mm long.



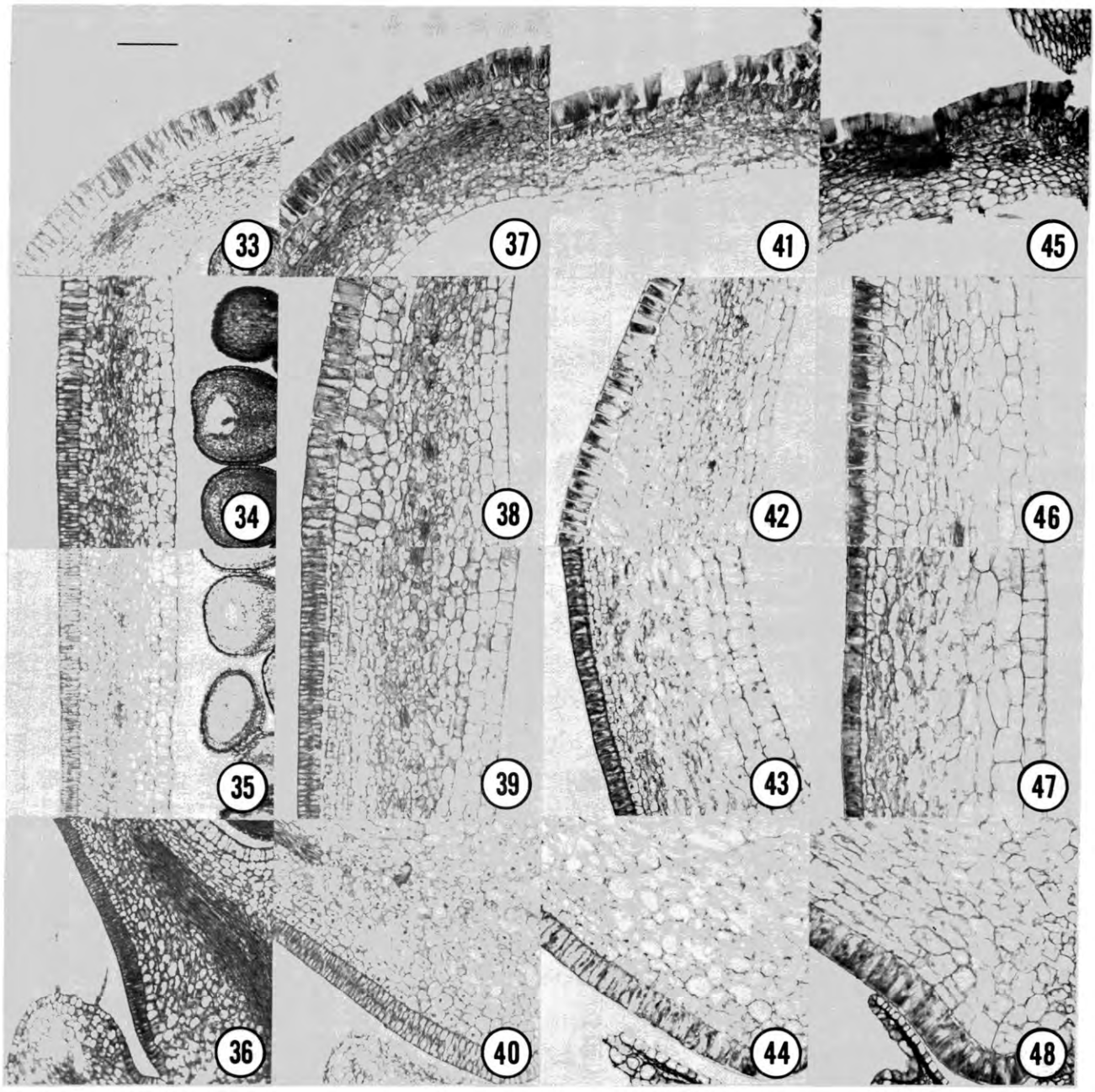
Figures 31-32
Longitudinal sections of the ovary of pollinated *Lychnis alba* flowers. (31) 36 hours after pollination, (32) 48 hours after pollination. Horizontal scale markings are 1.0mm long.

The first pollen tubes in the ''bridge'', i.e. the tissue connecting the styles with the placenta, appear 5-6 hours after pollination at 32°C and 200-400 have passed through the bridge 12-24 hours after pollination. Warmer weather hastens this process, cooler weather retards it (Dean, personal communication). It is reasonable to assume that ovary growth following pollination is initiated by cell enlargement promoted by growth substances carried by the pollen and/or whose production is stimulated by the presence of pollen tubes within ovarian tissue.

Although the small increase (14.2%) in ovary length during the interval between pollination and 24 hours thereafter may be attributed to a pollination stimulus, this factor alone would not appear to be sufficient to induce the remarkably large size increase noted during the next 24 hours.

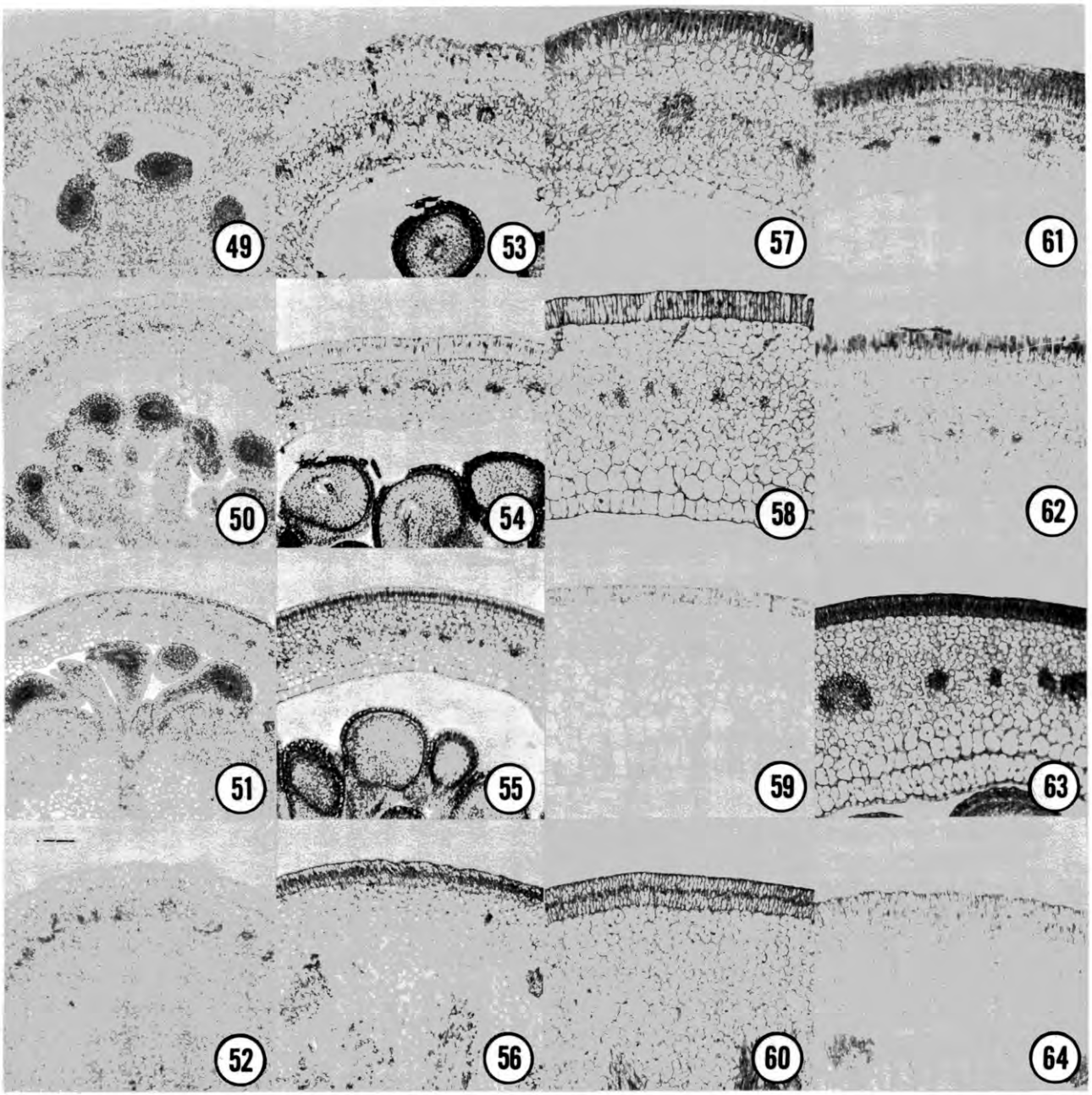
Devine (1950) stated that fertilization occurs 10-14 hours after pollination in *Lychnis* at an optimum temperature of 30°-32°C. Since growth between 12-48 hours after pollination amounted to over 100% one may assume that a second and much greater stimulus resulting from fertilization is the causative factor.

In *Lychnis,* therefore, the following may be inferred: Following pollination and as a direct result of it, extremely small quantities of growth substance either carried by the pollen tubes or formed in the ovary wall tissue in response to a stimulus provided by the presence of the pollen tubes initiates fruit development. Due to the small quantities of growth substance involved, the amount of fruit growth is relatively small. Following fertilization, the amount of growth substance increases in the developing fruit. The developing seeds may be the primary source of this substance. Assuming that synthesis and diffusion of such substance is not instantaneous a time lag would ensue while the material is made (activated) and moved from the developing seeds to the pericarp. This time lag would account for the second 10-12 hour period of limited growth. Once the growth substance reaches the pericarp, about 24 hours after pollination, a sharp rise in the rate of cell enlargement occurs with the resultant dramatic increase in fruit size.



Figures 33-48

Photomicrographs of longitudinal sections of the ovary of pollinated *Lychnis alba* flowers showing fruit wall structure at the points indicated. (33-36) 12 hours after pollination, (37-40) 24 hours after pollination, (41-44) 36 hours after pollination, (45-48) 48 hours after pollination. Figures 33, 37, 41, 45 are at point D, figs. 34, 38, 42, 46 are at point C, figs. 35, 39, 43, 47 are at point B and figs. 36, 40, 44, 48 are at point A. Horizontal scale marking is 0.1mm long.



Figures 49-64

Photomicrographs of cross sections of *Lychnis alba* ovaries showing wall structure at the points indicated. (49-52) unpollinated ovary approximately 3.5mm in length, (53-56) unpollinated ovary approximately 6.7mm in length, (57-60) 36 hours after pollination, (61-64) 48 hours after pollination. Figures 49, 53, 57, 61 are at point D, figs. 50, 54, 58, 62 are at point C, figs. 51, 55, 59, 63 are at point B and figs. 52, 56, 60, 64 are at point A. Horizontal scale marking is 0.1mm long.

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